Gezira j. of agric. sci. 10(2): 47-55(2012)

Effect of cytokinins and auxins on micropropagation of banana (*Musa* AAA) cv. Albeely by shoot tip explants

Mohammed H. A. Othman[1], Mohamed A. Ali[2] and Igbal A. Abdellatif[1]

[1] Faculty of Agricultural Sciences, University of Gezira, Wad Medani, Sudan

[2] Plant Tissue Culture, Agricultural Resarch Corporation, Wad Medani, Sudan.

ABSTRACT

This study was conducted in the plant tissue culture laboratory at the Agricultural Research Corporation (ARC), Wad Medani, Sudan, during the year 2011. The objective was to develop a micropropagation technique for banana, cv. "Albeely". Two cytokinins i.e. benzylaminopurine (BAP) and isopentenyladenine (2iP) were tested at 0, 2, 4, 6 and 8 mg/l each on micropropagation of the banana cv. Albeely. The results indicated that 4 mg/l was the best concentration for both cytokinins. Two experiments were conducted to determine the best combinations between 4 mg/l of both BAP and 2iP with different concentrations of indole butyric acid (IBA), i.e. 0, 0.2, 0.4, 0.6, 0.8 mg/l on micropropagation of banana. The results indicated that the best shoot morphogenesis was induced on either 4mg/l BAP combined with 0.8 mg/l IBA or 4 mg/l 2iP with 0.6 mg/l IBA.

INTRODUCTION

Micropropagation has played a key role in banana and plantain breeding programs (Vuylsteke *et al*., 1997). Among others, *in vitro* culture is of great advantage for mass propagation of various vegetative propagated crops. Plantlets produced through micropropagation methods have been found to establish faster, stronger, shorter production cycle and higher yields than those produced through conventional methods (Ortiz and Vuylsteke, 1996). Different *in vitro* propagation protocols, using different adenine-based cytokinins, have been used in several *Musa* spp. of divergent genomic constitution and ploidy (Vuylsteke, 1989). The proportion of auxin/cytokinin is a determinant factor for meristem formation

(George, 1993). Benzylaminopurine concentration range of 8.9 to 22.2 mM is recommended for *in vitro* propagation of *Musa* (Crouch *et al*., 1998). The use of rates more than this range induces increased rates of somaclonal variants (Trijillo and Garcia, 1996). This technique provides high rates of genetically uniform, pest and disease-free planting materials (Shirani *et al*., 2009). Therefore, the objective of this study was to develop a micropropagation technique for the banana cv. "Albeely".

MATERIALS AND METHODS

This study was conducted in the plant tissue culture laboratory at the Agricultural Research Corporation (ARC), Wad Medani, Sudan, during the year 2011. The banana cultivar used in this study was "Albeely". Suckers 5 to 7 months old of banana plants were obtained from the greenhouse of the Agricultural Research Corporation, Wad Medani, Sudan. Two experiments were conducted to test the effect of different concentrations of BAP and isopentenyladenine (2iP) at 0, 2, 4, 6 and 8 mg/l each on micropropagation of shoot tip explants of the banana cv. "Albeely" cultured on MS medium after 4 weeks.

Two experiments were conducted to determine the best combinations between 4 mg/l of both BAP and 2ip with different concentrations of indole butyric acid (IBA) i.e. 0, 0.2 , 0.4, 0.6 and 0.8 mg/l on micropropagation of shoot tip explants of the banana cv. "Albeely" cultured on MS medium after 4 weeks. All experiments were conducted using a completely randomized design with six replicates. Data were collected after 4 weeks. Analysis of variance was carried out using MSTATC computer program and Duncan's Multiple Range Test was used for means separation (MSU, 1993).

RESULTS AND DISCUSSION

Morphogenesis of shoot tip explants of the banana cv."Albeely" cultured on MS medium supplemented with different concentrations of BAP after 4 weeks.

The percentages of explants with shoots of the banana, cv. Albeely, induced by different concentrations of BAP after 4 weeks were not significantly different (Table 1). However, the number of shoots per explants was significantly different among BAP concentrations. The number of shoots per explants induced by the rage of 4-8 mg/l of BAP was comparable. On the other hand, low concentrations of BAP ranging between 0 and 2mg/l induced significantly lower number of shoots than the highest concentration of BAP. Hence, the optimum concentration of BAP for shoot induction on the banana cv. "Albeely" is 4 mg/l. Similar results were obtained by Vani and Reddy (1999). They reported that the best shoot proliferation of banana cvs. Dwarf Cavendish, Amruthapani, Tella Chak Kerakeli and Rubusta was induced on MS medium supplemented with 4 mg/l BAP. Obaid (2003) found that BAP at 5mg/l was the best concentration for induction and regeneration of shoots from both banana cvs. Williams Hybrid.31 and GN 1824. Cytokinins such as BAP and kinetin are generally known to reduce the dominance of apical meristems and induce axillary as well as adventitious shoot formation from meristematic explants in banana (Madhulatha *et al*., 2004).

Morphogenesis of shoot tip explants of the banana cv. "Albeely" cultured on MS medium supplemented with different concentrations of 2iP after 4 weeks.

The percentages of explants of the banana, cv. Albeely, with shoots were comparable on different concentrations of 2iP (Table 2). However, the number of shoots per explant was significantly different among 2iP concentrations. The highest number of shoots per explant was induced on 2iP concentrations which ranged between 4 to 8 mg/l. Low concentrations of 2iP ranging between 0 to 2mg/l induced

significantly lower number of shoots per explant than higher concentrations. The best concentration of 2iP for shoot induction was 4 mg/l. In contrast, Obaid (2003) found that 2.25mg/l 2iP was the best concentration for induction of shoot morphogenesis in both banana cvs. Williams Hybrid31 and GN1824.

Table 1. Morphogenesis of shoot tip explants of the banana cv. "Albeely" cultured on MS medium supplemented with different concentrations of BAP after 4 weeks

BAP concentration (mg/l)	Explants with shoots (%)	No. of shoots per explant
0	100	(3.2) 1.8 b
2	100	(5.5) 2.3 b
4	100	(9.0) 3.0 a
6	100	(9.5) 3.1 a
8	100	(8.8) 2.9 a
Mean	100	(7.2) 2.6
SE±	----	0.12
CV. (%)	----	18.6
Significance level	NS	***

Means in columns followed by different letter (s) are significantly different at P=0.05 according to Duncan's Multiple Range Test. Data between brackets are original. MS = Murashige and Skoog (1962).
NS,*** = Not significant and significant at $P \leq 0.001$, respectively.

Table 2. Morphogenesis of shoot tip explants of banana cv. "Albeely" cultured on MS medium supplemented with different concentrations of 2iP after 4 weeks

2iP concentration (mg/l)	Explants with shoots (%)	No. of shoots per explant
0	100	(2.8) 1.7 c
2	100	(3.7) 1.9 bc
4	100	(5.0) 2.2 ab
6	100	(6.0) 2.4 a
8	100	(5.5) 2.3 a
Mean	100	(4.6) 2.1
SE±		0.08
CV. (%)		15.4
Significance level	NS	**

Means in columns followed by different letter (s) are significantly different at P=0.05 according to Duncan's Multiple Range Test. Data between brackets are original. MS = Murashige and Skoog (1962). NS and ** = Not significantly different and significant at $P \leq 0.01$, respectively.

Elgorashi (1999) found that 8 mg/l 2iP was the best concentration for inducing shoots on banana cv. Dwarf Cavendish and the longest shoots were developed on 4mg/l 2iP. These differences in response to concentrations of 2iP might be due to genotypic differences.

Morphogenesis of shoot tip explants of the banana cv. "Albeely" cultured on MS medium supplemented with 4mg/l BAP and different concentrations of IBA after 4 weeks.

Table 3 shows that percentages of explants with shoots of the banana cv. Albeely induced by 4 mg/l BAP combined with different concentrations of IBA (0.0 - 0.8 mg/l) were not significantly different. However, the number of shoots per explant was significantly different among IBA concentrations. Significantly higher number of shoots per explant was induced on 0.8 mg/l IBA compared to the lower concentrations.

Table 3. Morphogenesis of shoot tip explants of the banana cv. "Albeely" cultured on MS medium supplemented with 4mg/l BAP and different concentrations of IBA after 4 weeks

IBA concentration (mg/l)	Explants with shoots (%)	No. of shoots per explants	
0.0	100	(7.8)	2.9 c
0.2	100	(8.3)	2.8 c
0.4	100	(11.3)	3.4 b
0.6	100	(11.2)	3.3 b
0.8	100	(14.3)	3.8 a
Mean	100	(10.6)	3.2
SE±	------		0.09
CV.%	------		11.3%
Significance	NS		***

Means in columns followed by different letter(s) are significantly different at P=0.05 according to Duncan's Multiple Range Test. Data between brackets are original. MS = Murashige and Skoog (1962); NS and *** = Not significantly different and significant at P≤ 0.001, respectively.

The number of shoots per explant induced on 0.4 and 0.6 mg/l of IBA were comparable, and were significantly higher than those induced on IBA concentration ranging from 0.0 to 0.2 mg/l. The best BAP/ IBA combination that induced the highest number of shoots per explant on the banana, cv. Albeely, was 4/0.8 mg/l, respectively. Dhed'a *et al*. (1991) reported that combinations of BAP with IAA or

IBA were effective for *in vitro* multiplication of bananas and plantains. Resmi and Nair (2007) reported high shoot multiplication, but with a reduction in the length of shoots in media with a combination of BAP and IAA in triploid cultivar of banana using inflorescence explants. The increased auxin level reduced the effect of the added cytokinin in promoting shoot proliferation, since for *in vitro* proliferation, a high cytokinin/auxin ratio has to be maintained (George, 1993).

Morphogenesis of shoot tip explants of the banana cv. "Albeely" cultured on MS medium supplemented with 4mg/l 2iP and different concentrations of IBA after 4 weeks.

The percentages of explants with shoots of the banana cv. Albeely induced by 4 mg/l 2iP combined with different concentrations of IBA (0.0 - 0.8 mg/l) were not significantly different (Table 4). However, the number of shoots per explant was significantly variable, among IBA concentrations. Significantly higher number of shoots per explants was induced on 0.6mg/l of IBA compared to zero and 0.8mg/l IBA. All other concentrations were comparable in number of shoots per explants. According to the results of this experiment, the 2iP/ IBA combination 4/0.6 mg/l, respectively, induced high number of shoots on the banana cv. "Albeely".

Table 4. Morphogenesis of shoot tip explants of the banana cv. "Albeely" cultured on MS medium supplemented with 4mg/l 2iP and different concentrations of IBA after 4 weeks

IBA concentration (mg/l)	Explants with shoots(%)	No. of shoot per explants	
0.0	100	(6.8)	2.6 b
0.2	100	(9.2)	3.0 ab
0.4	100	(9.7)	3.1 ab
0.6	100	(12.3)	3.5 a
0.8	100	(7.0)	2.6 b
Mean	100	(9.0)	3.0
SE±	------		0.10
CV.%	------		15.1%
Significance	NS		*

Means in columns followed by the different letter(s) are significantly different at P=0.05 according to Duncan's Multiple Range Test.
Data between brackets are original.,MS = Murashige and Skoog, (1962).
NS, * = Not significantly different and significant at P≤ 0. 05, respectively.

IBA and NAA are used in combinations with cytokinins for shoot proliferation of banana (Bhojwani and Razdan, 1983). Incorporation of a strong auxin in the media suppressed the shoot proliferation rates of the banana cultivars. The inoculation of AAA banana cultivars on hormone free medium (Talengera *et al*., 1994) showed root development with limited shoot multiplication. Thus, the results of their study indicated the existence of high levels of endogenous auxins and, therefore, the added auxins in the media resulted in increased total auxin levels resulting in high apical dominance and hence suppression of shoot proliferation. The increase in auxin level reduced the effect of the added cytokinin in promoting shoot proliferation since for in vitro proliferation, a high cytokinin/auxin ratio has to be maintained (George, 1993; Vuylsteke, 1989). In most cases, cytokinin/weak auxin (IBA) combinations induced higher shoot proliferation rates than cytokinin/strong auxin (NAA) combinations.

CONCLUSION

Micropropagation technique was successfully developed using shoot tip explants of banana cv. Albeely cultured on MS medium. BAP was more effective in shoot multiplication of the banana cv. Albeely compared to 2iP. The best BAP concentration for micropropagation was 4 mg/l. The combination of both BAP and 2iP with IBA improved the number of shoots per explant of the banana cv. Albeely.

REFERENCES

Bhojwani, S.S and M.K. Razadan. 1983. Plant Tissue Culture: Theory and Practice. Elsevier, Amsterdam.

Crouch, J. H., D. Vuylsteke and R. Ortiz. 1998. Perspectives on the application of biotechnology to assist the genetic enhancement of plantain banana (*Musa* spp.). Plant Biotechnology 1 (1): 1-12.

Dhed'a, D., F. Dumortier, B. Panis, D. Vuylsteke and E. De Langhe .1991. Plant regeneration in cell suspension cultures of cooking banana 'Bluggoe' cultivar (*Musa* spp. ABB group). Fruits 46: 125-135.

Elgorashi, E. S. 1999. Some *in vitro* Culture Techniques in the Propagation of the Dwarf Cavendish Banana. M.Sc. Thesis. Department of Horticultural Science. University of Gezira, Wad Medani, Sudan.

George, E. F. 1993. Plant Propagation by Tissue Culture. Exegenics Ltd., Edington, England. 574 pp.

MSU. 1993. MSTAT-C Microcomputer Program for the Design, Management and Analysis of Agronomic Research Experiments. Michigan State University, East Lansing, Michigan, USA.

Obaid, O. A. M. 2003. Micropropagation of Banana (*Musa* AAA) Clones Williams Hybrid 31, 189 and Grand Nain 1824. M.Sc. Thesis. Department of Horticultural Science. University of Gezira, Wad Medani, Sudan.

Ortiz, R. and D. Vuylsteke. 1996. Recent advances in *Musa* genetics, breeding, and biotechnology. Plant Breeding Abstracts 66:1355-1363.

Resmi, L. and A. S. Nair. 2007. Plantlet production from the male inflorescence tips of *Musa acuminata* cultivars from south India. Plant Cell, Tissue and Organ Culture 88: 333-338.

Shirani, S., F. Mahdavi, and M. Maziah. 2009. Morphological abnormality among regenerated shoots of banana and plantain (*Musa* spp.) after *in vitro* multiplication with TDZ and BAP from excised shoot tips. African Journal of Biotechnology 8(21): 5755-5761.

Talengera D., M. J. S. Magambo and P. R. Rubaihayo. 1994. Testing for a suitable culture medium for micropropagation of east African highland bananas. African Crop Science Journal 2:17-21.

Trijillo, I. and E. Garcia. 1996. Strategies for obtaining somaclonal variants resistant to yellow sigatoka (*Mycosphaerella muscicola*). InfoMusa 5 (2): 6-7.

Vani, R. K. and G. M. Reddy. 1999. Novel techniques in efficient micro-propagation of banana cultivars. Journal of Genetics and Breeding 53(3): 247-250.

Vuylsteke, D., R. Ortiz, R. S. Ferris and J. H. Crouch. 1997. Plantains improvement. Plant Breeding Review 14: 267-320.

Vuylsteke, D. 1989. Shoot-tip Culture for the Propagation, Conservation, and Exchange of *Musa* Germplasm. Practical Manuals for Handling Crop Germplasm *in vitro*. Rome, International Board for Plant Genetic Resources, Rome, Italy.

أثر السايتوكاينينات والاوكسينات على الإكثار الدقيق للموز (*Musa* AAA) صنف البيلي باستخدام النموات الطرفية

محمد حمود علي عثمان[1]، محمد احمد علي[2] و اقبال عبد القادر عبد اللطيف[1]

[1] كلية العلوم الزراعية، جامعة الجزيرة، واد مدني، السودان.

[2] هيئة البحوث الزراعية ، واد مدني، السودان.

الخلاصة

أجريت هذه الدراسة في معمل زراعة الأنسجة النباتية بهيئة البحوث الزراعية – واد مدني -السودان خلال العام 2011. بغرض تحسين الإكثار الدقيق للموز الموز صنف البيلي. تم تنفيذ تجربتين لاختبار أثر اثنين من السايتوكاينينات (بنزايل امينو بيورين وايزو بنتايل ادينين) بتراكيز مختلفة (صفر، 2، 4، 6،8 ملجم/لتر) لكل منهما. أوضحت نتيجة هاتين التجربتين أن 4 ملجم/لتر هو أفضل تركيز لكليهما. تم تنفيذ تجربتين لتحديد أنسب توليفة من التركيز الثابت من السايتوكاينينات (4 ملجم/لتر) مع تراكيز مختلفة من الاوكسين حمض أندول بيوترك (صفر، 0.2،0.4 ،0.6،0.8 ملجم/لتر). كانت أفضل توليفتين بين السايتوكاينينات والاوكسينات لتحفيز الإكثار الدقيق هي 4 ملجم/لتر من بنزايل امينوبيورين مع 0.8 ملجم/لتر من حمض أندول بيوترك و4 ملجم/لتر من ايزو بنتايل ادينين مع 0.6 ملجم/لترمن حمض أندول بيوترك.